【Grant-in-Aid for Scientific Research(S)】
Biological Sciences (Biological Sciences)

Title of Project : Molecular pathways leading to epigenome formation in mammalian germ cells

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ResearchArea : RNA Biology, Biochemistry, Molecular Biology
Keyword : Transposable elements, RNA silencing, epigenome, germ cells, PIWI

【Purpose and Background of the Research】

In RNA silencing pathways, small non-coding RNAs of 20–30 nucleotides in length function as specificity determinants for the repressive activities of Argonaute-containing effector complexes termed RISCs. Germline-specific small RNAs termed piRNAs are loaded onto germline-specific Argonaute proteins, or PIWI proteins, to yield piRISCs, in which piRNAs guide the repressive activities of PIWI proteins toward transposable elements (TEs) by means of base pairing to repress TE function in the gonad. It is becoming clear that piRISCs mediate TE silencing in the nucleus by directing specific epigenome formation at TE loci. However, the underlying mechanisms remain obscure.

The goal of this project is to understand molecular pathways leading to epigenome formation mammalian germ cells including primates through addressing three basic questions; how do nuclear piRISCs direct specific chromatin modifications at TE loci, what might be the targets of primate-specific piRISCs, and is it possible to develop s system that allows to direct specific epigenome formation at the locus of interest by artificially producing piRNAs?

【Research Methods】

Aim 1. To characterize nuclear piRISCs in mammalian germ cells. We have produced specific monoclonal antibodies against mouse Miwi2, a component of nuclear piRISCs that direct specific DNA methylation at TE loci on the genome. Using the antibodies, we will isolate nuclear piRISCs and address the question of whether nuclear piRISCs directly interact with DNA methyl transferases and other chromatin modification enzymes.

Aim 2. To characterize the forth PIWI present in primate genomes. Although Drosophila and mouse have three distinct PIWI genes, primates have four PIWIs. We have produced specific monoclonal antibodies that recognize the forth PIWI protein in common marmoset and human germ cells. We will immunopurify the forth PIWI-associated complexes and ask if piRNAs are present in the complexes.

Aim 3. To develop s system that allows us to direct specific epigenome formation at the loci of interest. We have recently identified *cis*-elements that direct piRNA production in germ cells. We will construct expression vectors that contain one of the *cis*-elements we identified and sequences that give rise to piRNAs, which in turn direct specific chromatin modifications at genes of interest.

【Expected Research Achievements and Scientific Significance】

With our approach we hope to produce an integrated picture of how the cell distinguish TEs from cellular counterparts and how it selectively silence them. We also hope to understand how defects in piRISC pathways lead to human disease including infertility.

【Publications Relevant to the Project】

1. "Biology of PIWI-interacting RNAs: new insights into biogenesis and function inside and outside of germlines" H. Ishizu, H. Siomi, & M.C. Siomi, ***Genes Dev*** **26**: 2361-2373 (2012)
2. "How does the Royal family of Tudor rule the PIWI-interacting RNA pathway?" M.C. Siomi, T. Mannen, & H. Siomi, ***Genes Dev*** **24**: 636-646 (2010)
3. "On the road to reading the RNA-interference code" H. Siomi, & M.C. Siomi, ***Nature*** **457**: 396-404 (2009)

【Term of Project】

FY2013-2017

【Budget Allocation】

167,800 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.siomilab.med.keio.ac.jp/